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Grazers superimpose humidity effect on stream biofilm resistance and resilience to dry-rewet stress

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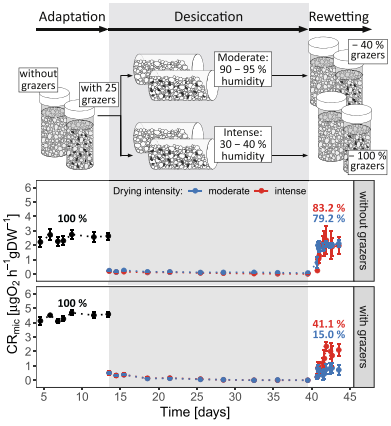
HIGHLIGHTS

- Grazed temperate stream biofilm response to increasingly episodic drying is unclear.
- We dried and rewetted biofilms in microcosms in the presence and absence of grazers.
- High humidity increased biofilm resistance to drying in the absence of grazers.
- The presence of grazers lowered the resistance and the resilience of biofilms.
- Food web interactions must be considered in predicting the metabolic effects of drying.

GRAPHICAL ABSTRACT



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ABSTRACT

Temperate low order streams increasingly experience intermittency and drying due to climate change. In comparison to well-studied Mediterranean streams, drying events in canopied temperate streams occur under higher ambient humidity which probably affects the metabolic response to drying. Previous work on drying sediments (in temperate streams) did not consider the interactions of trophic levels. We hypothesized that preservation of sediment moisture due to high humidity increases resistance to drying in temperate streambed biofilms and fast resilience of biofilm activity after flow resumption. We also expected the presence of macroinvertebrate grazers to modulate the biofilm response to dry-rewet stress. Following a two-level factorial design in 24 microcosms, we tested the effect of drying intensity (moderate and intense) and grazer presence and absence (*P. antipodarum*) on the activity of biofilm colonizing shallow hyporheic sediment. We measured the community respiration over a drying period of 27 days, a single rewetting event and a follow-up of three days. Grazer presence stimulated biofilm community respiration (CR_{mic}) in the permanently wet control, but decreased biofilm resistance to desiccation (<0.2% of pre-disturbed activity), regardless of drying intensity. In the absence of grazers, higher atmospheric humidity in moderately drying microcosms resulted in maintaining a film of adhesive water and low CR_{mic} (29% of pre-disturbed respiration) until the end of the drying period. After flow resumption, the CR_{mic} increased within 8 h, achieving 79–83% of pre-disturbed respiration (no grazers) and 15–41% (with grazers), respectively. Results show that short dry periods in temperate streams, even under high humidity, impact the streambed biofilm community negatively. The complex response and strong effect of grazer presence indicates that experiments including interactions of trophic levels and settings mimicking environmental factors during dry-rewet stress are needed.

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1. Introduction

Increase of water withdrawal and climate change are challenging the integrity and stability of freshwater ecosystems worldwide (Vörösmarty et al., 2000). Among the severe incipient and predicted impacts for fluvial ecosystems is the increasing occurrence of extended drought (Krysanova et al., 2008; Sabater et al., 2016; Sutherland et al., 2008; Ylla et al., 2010) and the concomitant geographical extension of intermittency in streams worldwide (Dieter et al., 2011). The ecological effects of drought depend on intensity, time of occurrence and duration. Prolonged drought leads to hydrological disconnection within river systems (Lake, 2003; Larned et al., 2010), cessation of flow (Dahm et al., 2003; Vázquez et al., 2015), desiccation of the stream bed and, thus, disrupted hyporheic flow (Febria et al., 2012). Streams and rivers in many regions shift from perennial to intermittent flow regimes by seasonal (i.e. Mediterranean streams) or irregular and rare (i.e. temperate streams) flow cessation (Costigan et al., 2017; Marxsen et al., 2010; von von Schiller et al., 2014) that have strong impacts on aquatic organisms and ecosystem functions (Döll and Schmied, 2012; Sutherland et al., 2008).

Research on Mediterranean streams reports that biogeochemical reactions, such as decomposition and mineralization of organic matter (OM), slow down upon bed desiccation (Larned et al., 2010; Romaní et al., 2013). Desiccation threatens the integrity and activity of sediment biofilms (Timoner et al., 2012) and decreases macroinvertebrate taxon richness (Stubbington and Datry, 2013). Partial adaptation to naturally occurring seasonal drying was found in several Mediterranean streams (e.g. Arce et al., 2015; Timoner et al., 2012; Ylla et al., 2010). Mediterranean streams show fast resilience within hours to days upon rewetting (Romaní et al., 2013) and often turn into net CO_2 sources to the atmosphere (Borken and Matzner, 2009; Gómez-Gener et al., 2016).

Temperate streams, however, are predicted to increasingly experience infrequent drought at irregular intervals (Costigan et al., 2017; Sutherland et al., 2008). As opposed to seasonal droughts in Mediterranean streams, there is still a lack of knowledge about the impact of irregular und unpredictable droughts in temperate streams (Lake, 2003; Marxsen et al., 2010; Pohlon et al., 2013). Many low order temperate streams are well shaded (Jones et al., 2010) and exposed to less prolonged droughts than Mediterranean streams (Borken and Matzner, 2009; Wanders and Van Lanen, 2015). The lower radiation and temperature result in higher atmospheric humidity near the drying bed, causing slower dewatering and some level of remaining sediment moisture even after a prolonged drought (Marxsen et al., 2010). Differences in the response between Mediterranean and temperate streams can be expected, since the intensity of drying and duration of dry periods is assumed to regulate the structural resistance and resilience of biofilms (Amalfitano et al., 2008; Gionchetta et al., 2018; Stubbington and Datry, 2013).

Another aspect that generally lacks profound investigation is the influence of grazing on the biofilm reaction to dry-rewet stress. Multiple studies verify strong top-down effects of grazers on aquatic biofilms (e.g. Heard and Buchanan, 2004; Moore et al., 2012; Rosemond et al., 1993; Shepard and Minshall, 2006; Wotton and Malmqvist, 2001). Biofilm structure, nutrient content and nutrient fluxes in biofilms are altered by grazing (Arango et al., 2009; Guo et al., 2009; Liess and Hillebrand, 2004; Moore et al., 2012), which also affects biofilm activity (Capps et al., 2015; Liess and Hillebrand, 2004; Mulholland et al., 1991). Grazing by snails severely reduces the content of extracellular polymeric substances (EPS) and thickness of lotic biofilms (Lawrence et al., 2002). Therefore, it can be assumed that the presence of grazers that generate a different type of biofilm, may alter the resistance and resilience of sediment biofilms to drying stress and subsequent rewetting.

We determined the interaction of invertebrate grazing (by *Potamopyrgus antipodarum,* Gray 1843) and two drying intensities (intense: 30–40% and moderate: 90–95% atmospheric humidity) on the functional resistance and resilience of a natural biofilm colonizing shallow hyporheic gravel. Here, we understand biofilm as the complex community of microbiota associated to the sediment surface, including bacteria, algae, extracellular substances and the inhabiting meiofauna such as protozoa (sensu Battin et al., 2016; Weitere et al., 2018). In the context of this paper, functional resistance is defined as the capacity of biofilm metabolic activity to withstand drying stress, and resilience as the capacity to recover after flow resumption (Shade et al., 2012; Stanley et al., 2004).

Colonized sediment was exposed in stream water perfused microcosms and four weeks of drying and subsequent rewetting were applied. We continuously determined community respiration as parameter of the biofilm activity. Following rewetting, we assessed the short-term functional recovery (2–69 h) which mainly reflects the activity and growth of organisms in the biofilm that survived the dry-rewet stress (Romaní et al., 2013; Ylla et al., 2010). We expected that grazers would affect the biofilm by stimulating the microbial activity during the aquatic adaptation period. The main hypotheses were: i) Resistance of the microbial community during the drying period is increased with high humidity (moderate drying) in a similar way for grazed and non-grazed biofilms, and ii) the resilience of biofilms is aggravated by the intensity of former drying and presence of grazers.

2. Methods

2.1. Study site and sediment sampling

Sediment samples in this study were collected from the groundwater-fed Waldbach stream, which is located near Bad Saarow, Brandenburg, Germany. The first order stream (Strahler, 1952) is fully canopied by beech (*Fagus sylvatica L.*) and alder (*Alnus glutinosa L.*). The stream sediment is composed mostly of sand and gravel and the occasional scattered rocks. The regional climate is temperate with an average annual precipitation of 576 mm (Wetterdienst, 2018). Although the uppermost reach is ephemeral, complete desiccation occurs on a very scarce basis. The last notably severe dry period affecting the lower reaches occurred in 2003 (personal communication, M. Mutz).

Individuals of *P. antipodarum* (>2 mm) and hyporheic sediment were sampled from the permanently inundated bed of a lower reach of the Waldbach stream on May 25, 2016 (UTM coordinates 52°25′N and 14°07′E). Sediment samples were taken beneath the top first centimeter of the streambed to a depth of about 3 cm, and carefully wet sieved in situ to extract fine gravel (2–6 mm) that was transported to the laboratory submerged in stream water. Snails were picked individually from the so-sieved grain fractions and individuals <2 mm and >6 mm were rejected. Snails were kept with spare sediment submerged in aerated stream water until the start of the experiment.

2.2. Microcosm set-up and experimental design

The gravel was transferred into microcosms (30 ml glass syringes, FORTUNA OPTIMA, Poulten & Graf GmbH, Wertheim, Germany) within 2 h after sampling. Each of the upright standing microcosms containing about 37 g fresh weight of sediment (31.6 g dry weight: DW) was filled up with filtered (10 μm) stream water and sealed with a silicone plug without headspace. The total volume of one microcosm amounted to 28.2 ml of which 13.1 ml was stream water and 15.1 ml was gravelly sediment. Twenty-five individuals of *P. antipodarum* were evenly distributed in the gravel in half of the microcosms. The chosen amount represented the abundance observed while sampling the sediments in the field. Each microcosm was constantly perfused from bottom to top with stream water (DOC: 3.83 mgC L^{-1}, DN: 0.34 mgN L^{-1}, SRP: 38.6 μgP L^{-1}) from an individual water reservoir (500 ml) by peristaltic pumps (205S/CA, Watson-Marlow GmbH, Ilsfeld, Germany, and IPC 12, Cole-Parmer GmbH, Wertheim, Germany). After perfusion through the sediment, the water drained from the microcosms through a small tube placed in the silicone plug and dropped down back into the reservoirs open to the atmosphere to ensure reaeration of the circulating water.

The water in the reservoirs was changed twice a week with fresh and filtered (10 μm) stream water. The microcosms and reservoirs were placed in a climate chamber at 17 °C and constant darkness. Each treatment (two drying treatments and one constant wet control, each with and without the presence of snails) was replicated four times, summing up to a total of 24 microcosms (Fig. 1).

All microcosms were perfused for 14 days to enable the adaptation of the disturbed communities to experimental conditions to serve as a reference for subsequent conditions of drying and rewetting. In the succeeding drying period of 27 days, 16 microcosms were cut off from the water circulation and the silicone plugs were removed. Sediment samples were drained for about 15 min to remove the water down to a water holding capacity (8.7% related to DW). All drying microcosms were placed in a separate dark climate cabinet at 23 °C to take into account the elevated temperature in the dry stream bed. The choice of 23 °C (compared to 17 °C for the wet control) was supported by data from the dry streambed of the uppermost reach of the Waldbach stream, where a maximum of 23.0 °C had been measured in 1 cm depth of bed sediments during the summer of 2016. Eight microcosms were exposed to the atmosphere of 30–40% humidity in the slowly ventilated cabinet (intense drying: ID) and eight microcosms were exposed to 90–95% water-saturated air that was generated by constantly blowing air through deionized water in a washing bottle (moderate drying: MD). After four weeks of drying, the microcosms were transferred back to the wet conditions at 17 °C by reconnecting them to the water circulation. Rewetting was realized by pumping filtered (10 μm) stream water from the bottom to the top through the sediment, simulating the natural rewetting process when streambed sediments are perfused by rising groundwater.

2.3. Community respiration of the microbial biofilm (CR_{mic})

The total respiration rates (CR_{tot}) during both wet phases (adaptation and rewetting) were assessed from the dissolved oxygen (DO, in mg L^{-1}) decrease in the water perfusing the sediment. An oxygen sensor spot (SP-PSt3-NAU, Precision Sensing GmbH, Regensburg, Germany; accuracy: ±0.04% at 20.2% O_2) on a glass fiber cable measured the DO concentration in the reservoirs from which water was pumped into the microcosms. Additionally, a needle-type oxygen microsensor (NTH-PSt1, Precision Sensing GmbH, Regensburg, Germany; accuracy: ±0.4% at 20.2% O_2) inserted into the microcosms through the silicon stopper measured the DO concentration after sediment perfusion. Both oxygen sensors had been calibrated with a two-point calibration (oxygen-free environment and air-saturated environment) before their use in the experiment. The difference in the DO concentrations measured before and after perfusion through the sediment served as a proxy for total respiration rates (CR_{tot}) and was given per g sediment DW ($\mu gO_2\ h^{-1} gDW^{-1}$) using water residence times in the sediments.

Respiration rates (CR_{tot}) during the drying process of sediments were assessed from measurements of CO_2 production using a gas analyzer (Ultraportable Greenhouse Gas Analyzer, LosGatos Research, California, USA). The microcosms were flushed for 30 s with fresh air of a known CO_2 concentration. After sealing them with gas-tight plugs, they were incubated at 23 °C for 5–6 h. An amount of 1–1.5 ml of gas was extracted from each microcosm with a gas-tight syringe and injected into the gas analyzer to measure the final CO_2 concentration. The CO_2 production was converted into DO consumption assuming a respiratory quotient of 0.85 (Bott, 1996). All respiration rates were

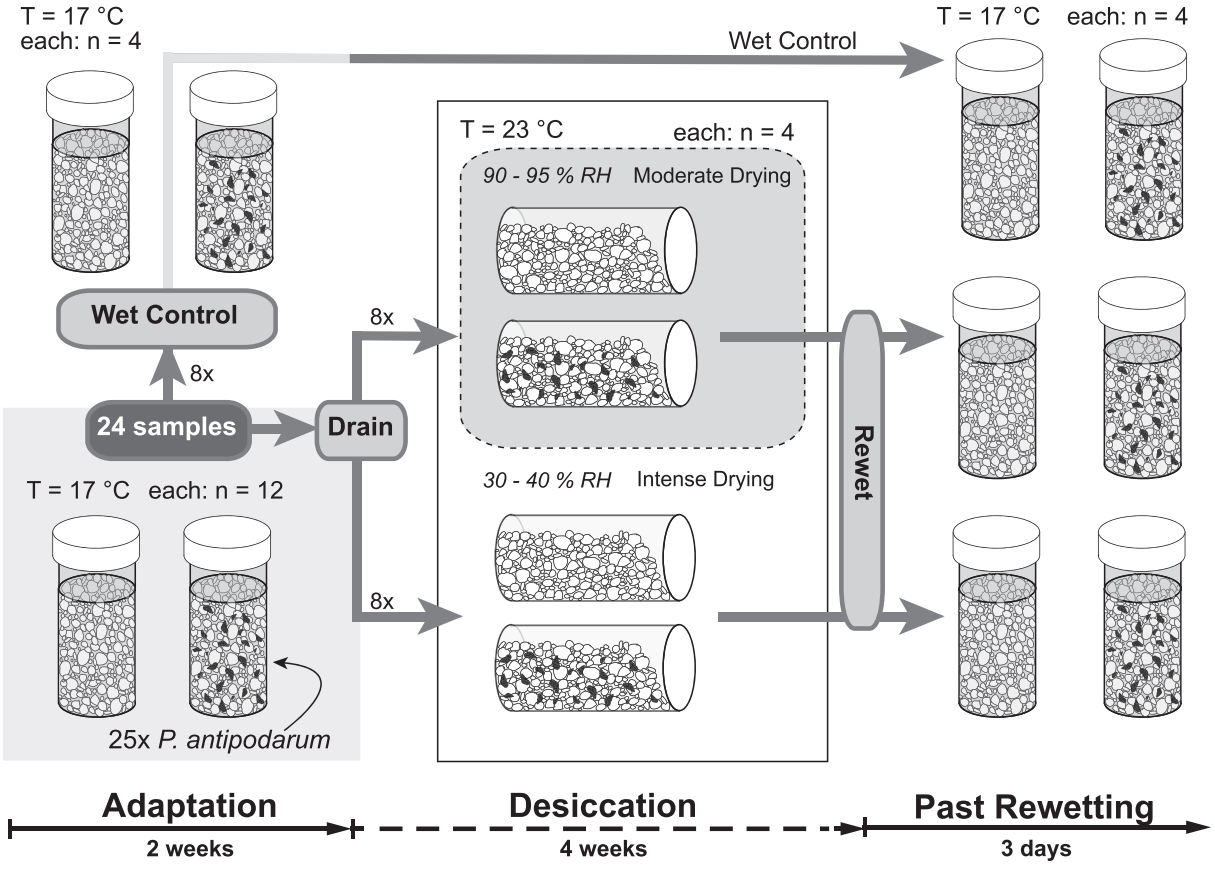

Fig. 1. Design of microcosm experiment. Twenty-four samples were collected (bottom left corner, gray box) and separated into two drying treatments groups (moderate and intense; RH = relative humidity) and one constant wet control (each $n = 8$). Black dots symbolize invertebrate grazers (*P. antipodarum*). The control remained in the same climate chamber at 17 °C, whereas drying treatments were exposed in a climate chamber at 23 °C.

adapted to a standardized temperature of 20 °C (Q_{10} factor of 2; Winkler et al., 1996). Samples were weighed after each measurement to assess the sediment moisture and progression of desiccation.

While CR_{tot} was generally measured twice per week, measurements were intensified following rewetting to assess the short-term reaction (2, 4, 6, 8, 19, 24, 30, 43, 48 and 69 h after rewetting). The biofilm community respiration (CR_{mic}) in samples with grazers was obtained by subtracting the separately measured respiration of grazers (CR_{graz}), microbial respiration from dead grazers (CR_{carc}) and microbial respiration associated with grazer feces (CR_{fec}) from the total respiration measured (CR_{tot}):

$$CR_{tot} = CR_{mic} + CR_{graz} + CR_{fec} + CR_{carc}$$

Therefore, CR_{mic} assessed the activity of the entire community inhabiting the sediment biofilm including protozoa and small meiofauna (sensu Weitere et al., 2018). Since we sampled the biofilm from the shallow hyporheic zone and performed the entire experiment in darkness, the potential contribution of algae was negligible and CR_{mic} addressed heterotrophic respiration only.

2.4. Grazer respiration (CR_{graz})

The grazer respiration (CR_{graz}) was measured in two parallel approaches (one for the wet control, one for the drying period), separate from the sediment-filled microcosms, to obtain the microbial respiration (CR_{mic}) of the sediment biofilm. Fifty individuals of *P. antipodarum* were each placed into six microcosms each containing three sterile glass beads (6 mm in diameter) to provide sufficient surface for locomotion. The grazer respiration was measured (DO consumption) and calculated similar to the sediment samples (temperature standardization).

Analogous to the water perfused microcosms with gravel and biofilm, a second set of grazers and glass beads in microcosms was exposed to the two drying treatments (MD and ID, 23 °C, 21 days of drought). The grazer respiration was measured (CO_2 production) and calculated similar to the sediment samples (respiratory quotient, temperature standardization). Grazer survival after drying was checked by placing snails on Petri dishes in stream water. Snails who attached their foot to the dish or moved from the initial position within 12 h were counted alive (Alonso and Castro-Díez, 2012; Poznańska et al., 2015; Winterbourn, 1970).

Grazer respiration (CR_{graz}) after rewetting was obtained by multiplying the constant grazer respiration rate in wet conditions by the proportion of living snails.

2.5. Respiration from grazer carcasses (CR_{carc})

Three glass flasks (46 ml) with 45 snails each were dried for 24 days at 23 °C and 30% relative humidity. Rewetting was performed with filtered (10 μm) stream water and the DO concentration was measured in closed flasks every 15 min with oxygen optodes according to the microcosm experiment. After 7 h, the flasks were opened for reaeration overnight and the water level was reduced to one third. On the next day, fresh water was added, and measurements followed for 2 h. The same procedure was repeated for a third day. Respiration per dead snail ($\mu gO_2\ h^{-1}Individuum^{-1}$) was multiplied by the respective number of dead snails in the microcosms during the rewetting phase only and then subtracted from the CR_{tot}. We assumed there was no respiration from dead grazers during desiccation.

2.6. Grazer feces production and feces-associated respiration (CR_{fec})

Forty-five snails were put on gravel colonized with biofilm in a beaker containing 250 ml of well aerated filtered (10 μm) stream water (three replicates). Feces produced by the snails were collected every second day from the bottom of the beakers with a pipette, sorted under a microscope (20× magnification), then dried and weighed to calculate the rate of feces production. The microbial respiration associated to the feces of the snails was measured separately from the suspension of freshly collected feces (0–24 h since defecation) in 2-ml respiration chambers.

We assumed accumulation of feces during the experiment and calculated the mean respiration rate by multiplying by the feces produced. We assumed zero feces production during the drying in MD and ID treatments. The number of snails which survived was taken into consideration when calculating feces production for each microcosm for the rewetting phase.

2.7. Statistical analysis

All data analyses were conducted in the R statistical environment (R Core Team, 2018, version 3.5.1). Statistical tests were considered significant at $p < 0.05$. The Shapiro-Wilk Test for normality was performed for $n = 84$ for data from the adaptation period and the samples did not differ from normal distribution (with grazers: $p = 0.66$, without grazers: $p = 0.41$) and were, therefore, considered as replicates hereafter. We used linear mixed-effect models (LMEM) using the lme4 package (Bates et al., 2015) to analyze the grazer influence during the adaptation period (LMEM: CR_{mic} ~ grazing + (1 + time|sample)) as well as the water content during the desiccation phase (LMEMs: k ~ grazing + (1|replicate) and k ~ drying intensity + (1|replicate)). CR_{mic} during desiccation was standardized to the respective value at the beginning of desiccation ($\frac{CR_{mic}}{CR_{mic,0}}$) to account for the variability between grazed and non-grazed biofilms established during the adaptation phase. Exponential regressions of the form $CR_{mic} = a \cdot e^{kt}$ were performed and differences among samples were assessed through the regression coefficients k ("strength" or "speed" of increase/decrease) using analysis of variance (ANOVA), testing for interaction between the grazer presence and drying intensity. The average respiration during the adaptation phase before desiccation was taken, depending on grazer presence, as a reference level. The rewetting phase was split into an immediate response (IR, 0–8 h after the addition of stream water) and a short-term resilience (STR, 19–69 h). The linear regression was fitted to the IR, whereas the STR measurements were averaged to compare treatments. Slope coefficients of linear regressions (IR) and mean CR_{mic} after the immediate response (STR) were compared separately using ANOVA. In the case of significant interaction, Tukey's HSD test was performed post hoc to find significant paired differences.

3. Results

3.1. Sediment water content during drying

The initial average gravimetric sediment water content after draining the pore water was 8.7% and was reduced during desiccation to 5.0% for MD and 1.4% for ID samples (Fig. 2). Drying was considered complete for both drying intensities, since the water content was constant after day 20 (80% of drying period). Exponential regression (R^2, MD: 0.99, ID: 0.91) showed that intensity of drying affected the sediment moisture since ID led to a faster decrease of the water content ($k = -0.08$) than MD ($k = -0.02$). The analysis of the exponential regression coefficients k proved that the presence of grazers did not affect the dynamics of sediment water loss ($n = 8$, $X^2 = 0$, $p = 1$), whereas the drying treatment did ($n = 8$, $X^2 = 18.89$, $p < 0.05$).

3.2. Grazer-, feces- and carcass-associated respiration

The number of grazers which survived in the microcosms clearly depended on the drying intensity. While all the snails survived in the wet control, MD drying caused a mean mortality of 40 ± 12% as

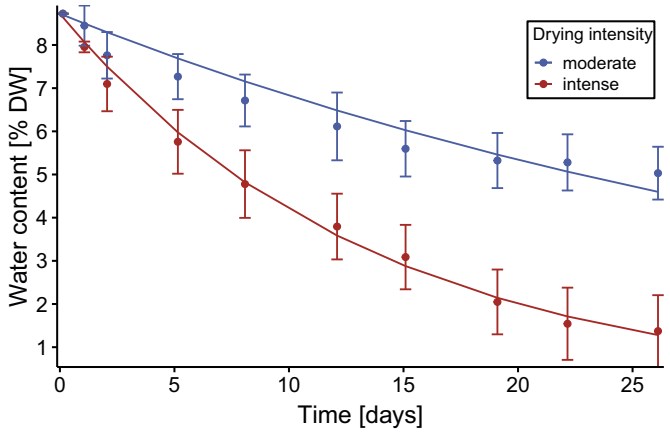

Fig. 2. Dynamics of the gravimetric sediment water content (as percentage of sediment DW) in moderate and intense drying treatments ($n = 8$, means ± SD). Colors indicate different drying intensities. Lines show fitted exponential models with a set intercept at y (x = 0) = 8.7%.

opposed to ID drying with 100% mortality. The 25 grazers during adaptation phase accounted for a constant respiration rate CR_{graz} of 0.67 ± 0.05 $\mu gO_2\ h^{-1}gDW^{-1}$ during the aquatic period (13.0% CR_{tot}). The snail respiration during drying was related to the drying intensity and varied over time. From a similar start level after draining (0.07 ± 0.01 $\mu gO_2\ h^{-1}gDW^{-1}$), respiration in the ID samples decreased within 2 d and was below the detection limit after two weeks of drought. It temporarily increased for 8 d to 0.17 ± 0.05 $\mu gO_2\ h^{-1}gDW^{-1}$ before decreasing in the MD samples. Both MD and ID samples had no detectable grazer respiration at the end as all the grazers in the microcosms were dead or had closed their operculum by the end of desiccation. During rewetting, the constant grazer respiration rate from the wet phase was adapted to a proportional number of snails that had survived in the microcosms. In MD sediments, the contribution of CR_{graz} to CR_{tot} rose to 25.3% (Table 1).

The average production of feces was 0.08 ± 0.01 $mg_{fec}\ d^{-1}$ per snail, amounting to 1.95 $mg_{fec}\ d^{-1}$ for a microcosm with 25 snails. The respiration associated with the feces accounted for 0.15 ± 0.03 $\mu gO_2\ h^{-1}\cdot mg_{fec}^{-1}$ before desiccation and 0.43 ± 0.01 $\mu gO_2\ h^{-1}mg_{fec}^{-1}$ after rewetting. The maximum CR_{fec} (related to sediment DW in the microcosms) before desiccation was found to be 0.12 $\mu gO_2\ h^{-1}gDW^{-1}$ (2.3% CR_{tot}) at the end of the adaptation period and increased to a mean of 0.39 ± 0.03 $\mu gO_2\ h^{-1}gDW^{-1}$ for rewetted samples. As the sediments contained a different number of snails after rewetting depending on the drying treatment, the part of CR_{fec} (relative to CR_{tot}) ranged between 5% and 25% (Table 1).

The microbial respiration of snail carcasses (CR_{carc}) following rewetting was low during the first 8 h and became stronger and consistent during the following two days. The CR_{carc} was, therefore, determined as 0.06 $\mu gO_2\ h^{-1}Individuum^{-1}$ for the first 8 h post rewetting and as 0.38 $\mu gO_2\ h^{-1}Individuum^{-1}$ for all subsequent measurements. Overall, CR_{carc} had the lowest relative contribution to CR_{tot} (Table 1).

3.3. Community respiration of microbial biofilm

3.3.1. Adaptation phase (effect of grazer presence)

Samples showed a stable level of respiration in the two-week adaptation period. The average respiration rate CR_{mic} for the entire adaptation phase amounted to 2.50 ± 0.70 $\mu gO_2\ h^{-1}gDW^{-1}$ for non-grazed and 4.40 ± 0.70 $\mu gO_2\ h^{-1}gDW^{-1}$ for grazed samples. A significant difference related to the grazer presence was confirmed ($n = 84$, $X^2 = 30.24$, $p < 0.05$).

3.3.2. Desiccation (functional resistance)

Results show that the major drop in CR_{mic} was due to disconnection from the water circuit, followed by the draining of pore water for about 15 min. Upon draining, the CR_{mic} was immediately reduced by 88.3% of the mean CR_{mic} during the adaptation phase (with grazers) and by 91.2% (without grazers), respectively (Table 2). Comparing the exponential regression coefficients of declining CR_{mic}, the biofilm response to drying was affected by both the presence of snails (ANOVA: $n = 4$, $F_{1,12} = 268.35$, $p < 0.05$) and the drying intensity (ANOVA: $n = 4$, $F_{1,12} = 18.08$, $p < 0.05$). The drying intensity was responsible for a difference in the reaction of CR_{mic} to drying only when the grazers were absent (Tukey's HSD test, $p < 0.05$). The MD samples without grazers maintained a small respiration level (29% of CR_{mic} related to the beginning of desiccation), whereas the ID samples dropped to 5% CR_{mic} (Fig. 3). However, the drying intensity showed no effect on the CR_{mic} in sediments containing grazers (Tukey's HSD test, $p = 0.47$). Grazed biofilms were observed to have the strongest reaction to drying (highest k) and declined to no detectable CR_{mic} by the end of desiccation (in the range of the detection limit, 0.01 $\mu gO_2\ h^{-1}gDW^{-1}$).

3.3.3. Rewetting (functional resilience)

After rewetting with stream water, we noted a surprisingly fast increase of respiration in previously dried samples to a respiration plateau after 19 h (Fig. 4). The immediate stimulation of CR_{mic} was observed as a linear response (0 h < t < 8 h) to fresh stream water for all sediments (Fig. 4). The increase of CR_{mic} was not influenced by the previous drying intensity and did not significantly differ from the wet control (ANOVA: $n = 4$, $F_{2,17} = 2.47$, $p = 0.11$). However, the stimulation of CR_{mic} was alleviated in the presence of grazers (ANOVA: $n = 4$, $F_{1,17} = 8.10$, $p < 0.05$). The STR (19–69 h) was compared to stable respiration levels during the adaptation period prior to dry-rewet stress (Table 3). The mean CR_{mic} at a stable level after the immediate response was termed a "degree of return", based on the approach of Todman et al. (2016). The grazer presence and drying intensity were involved in a complex interaction (ANOVA: $n = 24$, $F_{2,18} = 47.70$, $p < 0.05$, Fig. 4). The sediment biofilm without grazer presence achieved a relatively high degree of return (79–83%) regardless of the previous drying intensity (Tukey's HSD test, $p > 0.99$). However, the drying intensity did matter for the return of

Table 1
Breakdown of total community respiration (CR_{tot}) in grazed sediments into biofilm respiration (CR_{mic}), grazer respiration (CR_{graz}), respiration from grazer carcasses (CR_{carc}) and respiration associated to grazer feces (CR_{fec}) as absolute respiration in $\mu gO_2\ h^{-1}gDW^{-1}$ and percentage of CR_{tot} in brackets.

	CR_{mic}		CR_{graz}		CR_{fec}		CR_{carc}	
Drying intensity	bDes[a]	fRew[b]	bDes[a]	fRew[b]	bDes[a]	fRew[b]	bDes[a]	fRew[b]
Wet control	4.4 ± 0.7 (85.4%)	5.93 ± 0.51 (84.8%)	0.67 ± 0.05 (13.0%)	0.67 ± 0.05 (9.6%)	0.08 ± 0.02 (1.6%)	0.39 ± 0.01 (5.6%)	–	0
Moderate		0.66 ± 0.37 (41.8%)		0.40 ± 0.07 (25.3%)		0.40 ± 0.01 (25.3%)	–	0.12 ± 0.03 (7.6%)
Intense		1.81 ± 0.60 (73.3%)		0		0.36 ± 0 (14.6%)	–	0.30 ± 0 (12.1%)

[a] Before desiccation; mean respiration ($n = 84$) during adaptation period prior to desiccation.
[b] Following rewetting; mean respiration ($n = 24$) after immediate response ($t > 8$ h).

Table 2
Summary of exponential regression (adjusted R^2 and regression coefficient k) and comparison of beginning and end of desiccation values with the biofilm respiration (CR_{mic}) during the adaptation period. All values given are mean values ($n = 4$, if not stated otherwise).

Drying intensity	Grazers	R^2	k	Start of drying			End of drying				Adaptation period ($n = 84$)
				CR_{mic}		Water content	CR_{mic}			Water content	CR_{mic}
				$\mu gO_2\ h^{-1}gDW^{-1}$	%[b]	%[d]	$\mu gO_2\ h^{-1}gDW^{-1}$	%[b]	%[c]	%[d]	$\mu gO_2\ h^{-1}gDW^{-1}$
Intense	No	0.90	−0.11	0.20 ± 0.03	8	8.7	0.01 ± 0.008	0.4	5.0	1.65	2.50 ± 0.70
Moderate	No	0.90	−0.05	0.24 ± 0.02	9.6	8.7	0.07 ± 0.021	2.8	29.2	5.10	2.50 ± 0.70
Intense	Yes (0%[1])	0.95	−0.25	0.52 ± 0.13	11.8	8.7	<0.001	<0.1	<0.2	1.10	4.40 ± 0.70
Moderate	Yes (60 ± 12%[a])	0.89	−0.23	0.51 ± 0.05	11.6	8.7	<0.001	<0.1	<0.2	4.96	4.40 ± 0.70

[a] Percentage of snails surviving the drying period related to the starting number of 25.
[b] Related to CR_{mic} during the adaptation period.
[c] Related to start of drying.
[d] Water in microcosms as a percentage of sediment DW.

biofilm activity in the previous presence of snails (Tukey's HSD test, $p < 0.05$). The ID samples, where all grazers were dead, returned to 41% of their previous CR_{mic} activity, and regained a similar respiration as non-grazed biofilms (Tukey's HSD test, $p > 0.99$), whereas the grazed MD biofilms had the lowest degree of return of 15% (Table 3).

4. Discussion

4.1. Aquatic CR_{mic} is stimulated in the presence of grazers

Meeting our expectations, CR_{mic} was enhanced in the presence of snail grazers in concordance with literature (e.g. Danielopol, 1989; Majdi et al., 2016; Traunspurger et al., 1997). Top-down controlled organisms presumably allocate fewer resources to growth (Schimel et al., 2007), but show a stimulated respiration to maintain viable organismic functions (Hillebrand et al., 2002). Slos and Stoks (2008) reported a stress-induced increase in DO consumption under predation risk, which is concordant to our findings of stimulated CR_{mic} in grazed samples. Both feces (Guo et al., 2009) and adhesive grazer mucus (Peduzzi and Herndl, 1991) contain and release additional resources, such as C, N and P, which are known to stimulate CR_{mic} (Hillebrand et al., 2002; Rosemond et al., 1993).

4.2. Cessation of flow rapidly decreased community respiration

The harsh reduction of CR_{mic} by around 90% immediately following pore water drainage exceeded our assumptions of a strong reaction to dewatering. A key determinant in withstanding harsh conditions, such as drought, is the presence of temporary refuges (Lake, 2003, 2000; Robson et al., 2011; Timoner et al., 2012), such as damp sediment and hyporheic cavities (Robson et al., 2011; Sabater et al., 2016). In these, a small amount of water can be maintained, which is essential to the survival of aquatic organisms (Romaní et al., 2017). As our sediment consisted of fine gravel, the rather large pores drained fast and the low water content of 8.7% after drainage proved that microbes had little time to seek refuges. Furthermore, reduced access to resources (organic carbon and nutrients) by transfer from advective transport with the pore water flow to diffusive transport in the remaining water menisci and in the stagnant water film covering the biofilms may have caused the sudden decrease of CR_{mic} at the beginning of desiccation. Desiccation in natural temperate streams usually occurs over a larger time span (Larned et al., 2010). Starting with fragmentation in pools, the sediment moisture decreases and the stream bed dries out completely (Bogan et al., 2015; Sabater et al., 2016; Ylla et al., 2010). This gives reason to believe that CR_{mic} may drop more slowly in reality than in our experiment. Pohlon et al. (2013) showed that continuous dewatering by evaporation (instead of fast drainage by gravity) leads to a more gradual decline in extracellular enzyme activity. In contrast, abrupt and intense water stress leaves little opportunity for metabolic adaptation of organisms (Larned et al., 2010; Timoner et al., 2012). However, fast pore water drainage, similar to our experiment, does occur in streams, for example, at riffle crests and steeper banks and under anthropogenic acceleration of dewatering (i.e. water withdrawal or impounding).

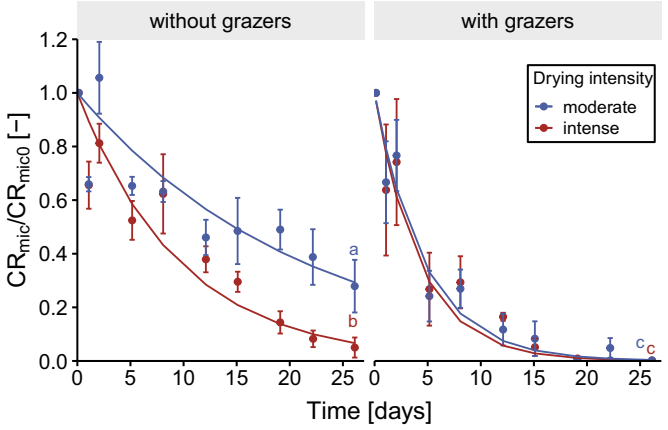

Fig. 3. Effect of drying intensity and grazer presence on the biofilm respiration (CR_{mic}) during desiccation. Drying intensity is indicated by colors. Decreasing CR_{mic} was standardized to the respective start value $CR_{mic,\ t=0}$ of the first measurement at $t = 0.13$ d. Lines show fitted exponential models with a set y-intercept at y = 1.0. The same letters indicate no significant difference for exponential regression between paired groups (Tukey's HSD, $p < 0.05$).

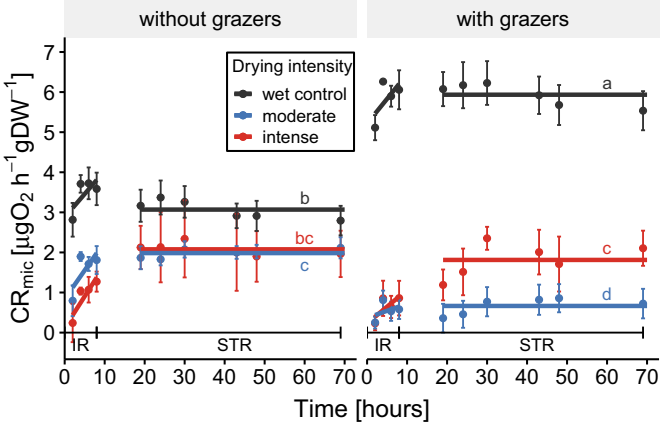

Fig. 4. Effect of drying intensity and grazer presence on the biofilm community respiration (CR_{mic}) following rewetting. Drying intensity is indicated by colors. Dots are mean respiration ($n = 4$) for each time increment, lines are linear increase during immediate response (IR, $t < 8$ h) and constant mean respiration over short-term resilience period (STR, $n = 24$, $t > 8$ h), respectively. The same letters indicate no significant difference for STR ($t > 8$ h) between paired groups (Tukey's HSD, $p < 0.05$).

Table 3
Comparison of biofilm respiration (CR_{mic}) before and after drying. Immediate response (IR, 0 h < t < 8 h after reperfusion with stream water) is given as the increase of CR_{mic} in time (slope of linear regression). Mean CR_{mic} for the short-term resilience (STR, 8 h < t < 69 h) is compared to the mean CR_{mic} during the adaptation period.

		CR_{mic}, Adaptation period ($n = 84$)	CR_{mic}, STR ($n = 24$)		$\frac{dCR_{mic}}{dt}$, IR ($n = 4$)
Drying intensity	Grazers	$\mu gO_2\ h^{-1}gDW^{-1}$	$\mu gO_2\ h^{-1}gDW^{-1}$	%[a]	$\mu gO_2\ h^{-2}gDW^{-1}$
Intense	No	2.50 ± 0.70	2.08 ± 0.71	83.2	0.16
Moderate	No	2.50 ± 0.70	1.98 ± 0.22	79.2	0.14
Intense	Yes	4.40 ± 0.70	1.81 ± 0.60	41.1	0.08
Moderate	Yes	4.40 ± 0.70	0.66 ± 0.37	15.0	0.01

[a] Related to CR_{mic} during the adaptation period (= "degree of return" sensu Todman et al. (2016)).

4.3. High humidity during sediment drying supports the maintenance of microbial activity in non-grazed biofilm

The desiccation process was demonstrated by exponentially decreasing sediment water content and was similar in the drying dynamics in a gravel-sand bed of outdoor experimental streams in temperate summer (Zlatanović et al., 2018). As expected, the two different degrees of air humidity led verifiably to two drying intensities. The dynamics of CR_{mic} during drying coincided clearly with the rate of water loss. The MD samples in non-grazed biofilms, that lost significantly less water than ID samples, could maintain 24% more of their incipient CR_{mic} when compared to ID samples. The effect of sediment moisture levels during desiccation has hardly ever been studied in freshwater science. Gionchetta et al. (2018) evidenced a high microbial sensitivity of freshwater sediment to minimal changes of the water content related to sediment depth. During desiccation, some important biogeochemical reactions, such as denitrification (Arce et al., 2015) and decomposition and mineralization of organic matter (Amalfitano et al., 2008; Dieter et al., 2011; Larned et al., 2010) slow down considerably. A higher moisture content in soil is known to generally entail stimulated soil respiration by microbes (Davidson et al., 2000; Kukumägi et al., 2014; Orchard and Cook, 1983; Suseela et al., 2012).

Any microbial adaptation to hydric stress requires resources (Schimel et al., 2007) and an electron acceptor (such as O_2) to metabolize these. As our microcosms were open to the atmosphere and drained pores filled with air, aerobic bacteria had sufficient access to oxygen. However, with declining bed moisture, nutrients and organic substrates become increasingly less available due to a shift from advective transport to the slower process of diffusion (Borken and Matzner, 2009; Wang et al., 2015). Diminishing resources impede the microbial production of protective molecules, such as osmolytes, to react to increasing osmotic stress (Borken and Matzner, 2009; Moyano et al., 2013; Schimel et al., 2007). The higher activity in MD samples suggests that biofilms could have had better access to resources as desiccation continued. A thin water film over and water in the biofilm presumably provided a diffusion medium for organic substrates and facilitated the function of exoenzymes and locomotion of microbes.

4.4. Intolerance of P. antipodarum to drying stress

The high mortality of grazers found in our experiment is backed by several studies which tested the desiccation tolerance of *P. antipodarum* (e.g. Alonso and Castro-Díez, 2012; Bennett et al., 2014; Duft et al., 2003; Lancaster and Ledger, 2015; Winterbourn, 1970). Survival times of 4–7 days are reported (Alonso et al., 2016; Poznańska et al., 2015; Wood et al., 2011) that are well below the duration of the drying phase in our experiment. Our observations of a lower mortality under high humidity drying are affirmed by Richards et al. (2004) who state a 50% higher chance of survival if desiccation occurs under 90–100% humidity compared to 20–25% humidity.

4.5. Snail grazers superimpose humidity effects and lower the drought resistance of biofilms

Our hypothesis of the influence of snail grazers on the biofilm resistance to drying was confirmed, as grazed biofilms showed a significantly stronger decrease in CR_{mic} and lower resistance to drying than non-grazed biofilms, even disregarding the humidity. We could not measure biofilm structure and thickness in our experiment, but grazing snails are known to inhibit the accumulation of biomass and to remove layers of EPS and senescent cells (Sabater et al., 2007). In stream biofilms cultivated on polycarbonate slides, grazing snails even removed the entire EPS superstructure reducing the biofilm to a thin layer of bacterial cells (Lawrence et al., 2002). Layers of EPS enhance hydration and buffer cells against moderate desiccation (Or et al., 2007; Romaní et al., 2013). The potential of thicker biofilms to attenuate drying stress can explain why the effect of drying intensity on CR_{mic} was just observed in non-grazed samples. The sediments with grazed and most likely thinner biofilms obviously responded with maximal sensitivity even at moderate hydric stress. High grazer mortality and fast decreasing CR_{mic} may further be correlated, as rising water stress prevented the hydrolysis of highly nutritious snail mucus (Guo et al., 2009), which then became inaccessible to extracellular enzymes and bacteria (Peduzzi and Herndl, 1991). Since we sampled sediment and biofilms from a natural stream, micrograzers such as ciliates likely existed in all treatments. However, other than snails, small micrograzers are not able to fully control the structure of river biofilms (Gücker and Fischer, 2003; Weitere et al., 2018). Hence, the effects found in our experiment are most likely limited to grazing by bigger organisms such as snails.

4.6. Resumption of activity after rewet

Rewetting provokes a prompt and intensified resumption of biogeochemical reactions (McClain et al., 2003; Meisner et al., 2013; Timoner et al., 2012; Wang et al., 2015), leading to a fast recovery of viable ecological functions (Ylla et al., 2010). The immediate respirational reaction of dried biofilms in our experiment is strong evidence for the presence of still viable extracellular enzymes which can be stable in dry soils and sediments for weeks (Gionchetta et al., 2018; Perez-Mateos et al., 1991; Zoppini and Marxsen, 2011), and the presence of dormant cells that rapidly reactivate their metabolism (Schimel et al., 2007).

Rehydration of a dried soil evokes a respiration pulse (Birch, 1958; Orchard and Cook, 1983), which often exceeds the level of the constant wet control. We did see a rapid onset of CR_{mic} within the first 8 h, similar to other studies from streams (e.g. Marxsen et al., 2010; Sabater et al., 2016; Timoner et al., 2012; von Schiller et al., 2015). However, an overall positive effect of the dry-rewet stress on the immediate respirational response, such as the pulse found in soil (Meisner et al., 2015; Pietikäinen et al., 2005), did not occur in the stream sediments. The respiration pulse in soil is based on the availability of nutrients and assimilable substrates originating from lysis of dead cells and desorption of DOC during rewetting (Guo et al., 2014; Kaiser et al., 2015). Compared to slow capillary water saturation in soil, rewetting in stream bed

sediments occurs faster due to larger pores and higher hydraulic conductivity (Arce et al., 2019) and flow resumption mostly sets on instant pore water flow, as also simulated in our experiment. Hence, nutrients and assimilable substrates are rapidly flushed downstream (Merbt et al., 2016), removing the sources for increased biofilm respiration.

Confirming our hypothesis, the MD and ID biofilms did not achieve full resilience by the end of our experiment (≤83% degree of return, sensu Todman et al. (2016)). This suggests that the mortality of cells and lasting damages from the dry-rewet stress reduced the microbial community and its metabolic potential, as reported by Marxsen et al. (2010). Our results are consistent with the findings in experimental streams (Acuña et al., 2015; Zlatanović et al., 2018), where heterotrophic respiration did not return to pre-drying levels after rewetting.

Surprisingly, the drying intensity that affected the level of functional resistance during drying did not show any influence on the degree of return in non-grazed biofilms after rewetting. This decoupling of functional resistance and resilience has been observed before (Muñoz et al., 2018) and may be due to variable reactions of different microbial groups to water stress. Several researchers report a shift of sediment community composition towards water stress resistant groups (e.g. Gram-positive bacteria) (Marxsen et al., 2010; Pohlon et al., 2013; Schimel et al., 2007). It is likely that the low activity we measured during desiccation was provided largely by these groups. Stress resistant groups are known to relate positively to slight changes in sediment water content (Pohlon et al., 2013), as observed in our experiment. The much higher resilient activity after pore water flow resumption, however, could have possibly been defined by the fast reestablishment of dormant aquatic cells. This was already proposed by Simon et al. (2016), who did not observe a shift in composition of the microbial community after a one-month drought event.

4.7. The complex effect of grazers on biofilm resilience

The influence of snail grazers on post-rewetting biofilm functional resilience was more complex than expected. The drying intensity, being insignificant for resistance, affected the short-term resilience, supporting our proposal of decoupled functional resistance and resilience. Surprisingly, the grazed ID biofilms aligned to the same degree of resilience as non-grazed biofilms. This may indicate a shift in the biofilm community composition that points to the possible microbial adaptation for future recurring wet-dry cycles (Febria et al., 2012; Goldman et al., 2017). Our assumption that surviving grazers in MD samples respired to their pre-drying level after rewetting may have been an overestimate leading to an exaggeratedly reduced CR_{mic}. According to Alonso et al. (2016), grazers that have experienced severe desiccation may suffer subsequently from prolonged organismic damage regarding their reproductive capabilities and ecological fitness. However, given the hypothetical case that all snail grazers were dead in the MD samples, therefore, having the same CR_{graz} and CR_{carc} as in ID samples, the degree of return was still significantly lower compared to ID microcosms. Contrarily, it is also possible that the few remaining grazers in the MD microcosms fed excessively on biofilm to recuperate from drought stress. In this case, we would have underestimated the CR_{graz} and the CR_{mic} would be even lower. Therefore, grazer activity alone cannot explain the difference in the degree of return between MD and ID. The rate and extent of activity resumption of invertebrates immediately after rewetting and in the short-term resilience period still remain a knowledge gap and are subject to assumptions until further investigations have been made. The higher degree of return observed in ID biofilms may thus be concealing a larger, longer-lasting impact of the dry-rewet stress on the biofilm community.

5. Conclusion

In this study, we simulated a dry-rewet disturbance in the shallow sediment of a temperate stream. To the best of our knowledge, this is the first time that the effect of different degrees of atmospheric humidity on streambed moisture and, in turn, sediment microbial activity has been investigated. As streams dry out from top to bottom, the hyporheic sediments serve as a moist refuge for the stressed invertebrate and bacterial communities. We found that higher sediment moisture, as in shaded and temperate conditions, may not completely hinder the negative impacts of desiccation in streams. However, it can delay drastic and harmful complete desiccation by preserving just an essential level of water during dry periods. Fractional rewetting (i.e. rainfall), which often interrupts summer heat periods in temperate regions, could thus contribute to maintaining a hyporheic water film (Arce et al., 2015; Gionchetta et al., 2018). Therefore, the biofilm in the sediments of temperate streams with higher moisture may have a better chance of withstanding desiccation, than biofilms facing harsher drying conditions such as in Mediterranean climate.

However, we showed that the microbial response to dry-rewet stress varies with the presence and absence of grazing snails, probably driven by differences in the architecture, the biomass, and the EPS content of the biofilm caused by grazing. The biofilm, under predation pressure by grazers, is threatened with losing the protective polymer layer and associated vital water film which help to cope with drought periods. This effect is stronger during drying than the drying intensity (atmospheric humidity), so that the resistance of naturally top-down controlled biofilms deteriorates, although grazing generally stimulates the biofilm activity under inundated conditions. The resilience to dry-rewet stress showed a strong decoupling from the resistance to both the presence and absence of grazers, pointing to different fractions of the microbial community that provide the activity during dry stress and post rewetting.

Our findings confirm the severe impact of irregular und unpredictable drying on the metabolism in temperate streams. We found evidence that high humidity can possibly mitigate the drying stress for non-grazed biofilms, supporting the importance of riparian shading to reduce the drought impact on temperate streams. Surprisingly, intensive grazing by snails that is common in many streams reversed the beneficial effects of high humidity. Therefore, further investigations are needed to disentangle the factors controlling functional resilience. We need to consider the full complexity of stream ecosystems, including their trophic interactions, before reliable models and predictions on the response of temperate streams to dry-rewet stresses can serve as a basis for management.

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